THE OLIGOSACCHARIDES OF SOME JAPANESE LEGUMES. I.

Preliminary Experiments by Paper Chromatography.

Teiiti NARASAKI (Laboratory of Agricultural Products Technology)
and Sin'itirô KAWAMURA (Laboratory of Biological Chemistry)

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INTRODUCTION

Concering the oligosaccharides of the soybean, KAWAMURA [1] reported that the soybean contained saccharose, raffinose, and stachyose. A sample of the soybean contained them in the amounts of 3.7, 1.0, and 3.2%, respectively [2]. HASEGAWA et al. [3] reported that the soybean, kidney bean, asparagus bean, and pea showed the presence of all these three oligosaccharides by paper chromatography. KAWAMURA, TUBOI, and NAKAMURA [4] could ascertain that also the broad bean showed the presence of the same three oligosaccharides. Now paper chromatography was applied to see the distribution of the oligosaccharides in the five legumes, *i.e.* adzuki bean, mung, kidney bean, asparagus bean, and sword bean.

MATERIALS

The five legumes used as the sample were as follows:

1. Adzuki bean, *Phaseolus chrysanthos*, "azuki" in Japanese.
2. Mung, *P. aureus*, "ryokutô".
3. Kidney bean, *P. vulgaris*, "ingen-mame".
4. Asparagus bean, *Vigna sesquipedalis*, "sasage".
5. Sword bean, *Canavalia gladiata*, "natamame".

They were all purchased on the market, were in excellent conditions, and were stated to have complete germinating abilities. They are the same legumes as used in the study of starches by KAWAMURA, TUBOI, and HUZII [5], who gave the analyses reproduced in Table 1.

Table 1. Analyses of legumes used [5].

	Moisture	Crude protein	Crude fat	N-free extract	Crude fiber	Ash
1. Adzuki bean	14.55	21.27	0.80	52.40	7.95	3.03
2. Mung	14.43	22.03	0.91	54.98	5.02	2.63
3. Kidney bean	14.59	22.05	1.44	53.22	4.94	3.25
4. Asparagus bean	14.38	21.27	1.34	54.45	5.76	2.80
5. Sword bean	14.81	24.06	1.38	52.32	4.61	2.82

These legumes were dehulled and pulverized. The sugars were extracted from 20 g of them with 200 ml 50% ethanol by allowing to stand for about 2.5 hours at 70° with occasional shaking. The extract was filtered with sintered glass filter, the filtrate was evaporated to dryness *in vacuo* below 50° on a water bath, and the resulting thin film of the sugars was taken up in 5 ml of 50% ethanol. After filtration this is ready to be applied to paper chromatography.

METHOD

Apparatus. One-dimensional chromatographic apparatus of Tôyô Filter Paper Co. (Tokyo).

Paper. Paper strip of Tôyô No. 50 (2 cm × 40 cm).

Two solvent systems were used:

Solvent A. A mixture of *n*-butanol, acetic acid, and water (4 : 1 : 2, v/v) was shaken in a separating funnel and the upper layer was used after allowing to separate into two layers.

Solvent B. A mixture of phenol and water (17 : 3, wt/wt).

Color reagents. Ammoniacal silver nitrate [6] and *p*-anisidine phosphate [7] were employed.

Procedure. The sugar solution, 5-10 μl, was spotted on paper strip with a micropipette. The chromatograms were developed for about 20 hours with solvent A and about 24 hours with solvent B at constant temperature of 22±1° C. The double development was made with solvent A. Following the development of the chromatogram, the sugars on the strips were revealed by use of the spray reagents and each spot was identified by comparing their colors and R_f values with those of standard sugars.

RESULTS AND DISCUSSION

As shown in Tables 2 and 3, all samples of legumes contained no reducing sugar. The presence of saccharose, raffinose, stachyose, and one or two more unidentified oligosaccharides was found. Sword bean seemed to contain another unidentified oligosaccharide over the spot of raffinose on the chromatogram. According to DUPERON [8], the distribution of saccharose, raffinose, and stachyose is considerably wide.

The oligosaccharides corresponding to lower R_f values than the R_f of stachyose not detected in the cases of the soybean and broad bean might be pentasaccharide and hexasaccharide. COURTOIS, *et al* [9]. found verbascose or trigalactosidosaccharose in the seeds of lentil, alfalfa, and vetch (all belonging to Leguminosae). They[10] further found the existence of ajugose or tetragalactosidosaccharose in vetch seeds.

Thus it is very probable that the mung contains these two higher oligosaccharides (verbascose and ajugose), sword bean contains verbascose, and the adzuki bean, kidney bean, and asparagus bean contain either or both of them.

The above findings were reported by KAWAMURA.[11] It is very interesting to note that very recently GIRI[11] reported the existence of saccharose, raffinose, stachyose, and verbascose in green gram (*Phaseolus radiatus*), which is understood to be identical with mung in this report.

Table 2. Detection of oligosaccharides by paper chromatography with solvent A, BuOH-AcOH-H_2O (4:1:2).

R_f of standard sugar		R_f and size of spots from samples				
		1. Adzuki bean	2. Mung	3. Kidney bean	4. Asparagus bean	5. Sword bean
Saccharose	0.24	0.25 ##	0.24 ##	0.24 ##	0.24 ##	0.24 ##
?						0.15 ±
Raffinose	0.13	0.13 +	0.12 +	0.13 +	0.13 +	0.13 +
Stachyose	0.07	0.07 +	0.07 +	0.06 ##	0.06 ##	0.07 +
(Verbascose ?)	—	0.03 +	0.04 +	0.03 +	0.03 +	0.04 +
(Ajugose ?)	—		0.02 +			

Table 3. Detection of oligosaccharides by paper chromatography with solvent B, PhOH-H_2O (17:3).

R_f of standard sugar		R_f and size of spots from samples				
		1. Adzuki bean	2. Mung	3. Kidney bean	4. Asparagus bean	5. Sword bean
Saccharose	0.34	0.33 ##	0.34 ##	0.32 ##	0.34 ##	0.32 ##
Raffinose	0.23	0.22 +	0.22 +	0.22 +	0.23 +	0.22 +
Stachyose	0.12	0.12*##	0.09*##	0.10*##	0.10*##	0.10*+

* The spot showed tailing toward lower R_f value.

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豆類の少糖類について

I ペーパークロマトグラフィーによる少糖類の検出

楢崎丁市，川村信一郎

豆類の少糖成分としては，サッカロース，ラフィノース，スタキオースが知られている[1-4]．川村[5]は日本産の各種豆類の澱粉について比較生化学的研究を行っているが，そこで使用されている豆類の中から5種を選び，その少糖類をペーパークロマトグラフィーにより検索した．

使用したのは，アズキ，緑豆，インゲン豆，ササゲ，ナタマメの5種で，脱皮，粉砕後，50%温エタノールで少糖類を抽出した．展開はブタノール-酢酸-水（4：1：2）（2回）とフェノール-水（17：3）で行い，アンモニア性硝酸銀と*p*-アニシジンで発色させた．

単糖類はどの試料からも検出されなかった．サッカロース，ラフィノース，スタキオースはすべての試料から一様に検出され，Duperon[8]の報告が確認された．さらにスタキオースよりR_f値の小さい糖が1～2種と，ナタマメではラフィノースよりR_f値の大きい未確認糖1種が検出された．

Courtois[9]等は扁豆，ムラサキウマゴヤシ，及びカラスノエンドウからトリガラクトシドサッカロース，ベルバスコース，を発見し，さらに彼等[10]はテトラガラクトシドサッカロース，アジュゴース，をカラスノエンドウから発見している．これらの糖標品が入手できなかったので確認はできなかったが，筆者等の検出したスタキオースよりR_f値の小さい2種の糖はおそらくベルバスコースとアジュゴースであろう．従つて緑豆はこれら2種を，ナタマメはベルバスコースを，他のアズキ，インゲン豆，及びササゲはこれらの内いずれか1種或は両方を含んでいるように思われる．最近行われた国際酵素化学シンポジウムでGiri[12]は*Phaseolus radiatus*の種子中にサッカロース，ラフィノース，スタキオース，の他にベルバスコースがあり，これらの少糖類がトランスガラクトシデーションによりサッカロースから生ずることを報告したが，この豆は本報の緑豆に一致する事がわかった．